

In view of the above, it is believed that this application is in condition for allowance and a Notice to that effect is respectfully requested.

Respectfully submitted,

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## **APPENDIX**

### **VERSION WITH MARKINGS TO SHOW CHANGES MADE**

#### **IN THE SPECIFICATION:**

#### **Proposed Amendments To The Specification Showing Deletions And Insertions.**

#### **Paragraph at Page 17, line 19**

5' -ACGGTTGTTGAATTACCGGTGTTAATAGAG- 3' SEQ ID NO:4

#### **Paragraph at page 20, line 2 to Page 21, line 10**

[Method 1] The upstream region of the pra2 gene was amplified with a series of upstream region primers containing a HindIII recognition sequence at the 5' end and a primer containing the NcoI recognition sequence corresponding to the start codon ATG of the pra2 gene (NcoI primer: 5' - GGTCCATGGTCTTGTCAAGATC- 3') (SEQ ID NO:5). Deletion clones for linker scanning were constructed with primers containing a change of 6 nucleotides corresponding to the PstI recognition sequence in the upstream region primers (LS constructs). The amplified fragments were subcloned into the EcoRV site of pZEr0-2.1 (Invitrogen) and digested with HindIII and NcoI. HindIII-NcoI fragments were separated by electrophoresis to recover DNA fragments of interest using a DNA extraction kit (Pharmacia). The plasmid pBI221-LUC containing a luciferase gene (described in "Experimental Protocols for Observing Plant Cells", pp. 199-200, Shujunsha) was digested with HindIII and NcoI, and the DNA fragments was linked to the above recovered DNA fragments. The nucleotide sequences of the subcloned DNA fragments were determined to be

identical with the sequences of corresponding domains in the 5' upstream region of the pra2 gene. This method 1 was used to construct the following clones using amplification primers shown in parentheses:

PL1 (5' -GGGAAGCTTTAAAGGCAAGGG- 3' (SEQ ID NO:6) and NcoI primer),  
PL3 (5' -ACGTAAAGCTTAAAAATTCACCC- 3' (SEQ ID NO:7) and NcoI primer),  
PL4 (5' -AAATAAAGCTTAAAAGTAACACATA- 3' (SEQ ID NO:8) and NcoI primer), PL4B (5' -AAATAAAGCTTAAAAGTAACACATA 3' (SEQ ID NO:8) and 5' -GTACTGCAGTCAGACATGATTAACAAG- 3') (SEQ ID NO:9) , PL5 (5' -AAAGAAGCTTGGTAGCCCAAACAA- 3' (SEQ ID NO:10) and NcoI primer), LS1 (5' -AAGCTTctgcagGGATTTTACAGTAATAAA- 3' (SEQ ID NO:11) and NcoI primer), LS2 (5' -AAGCTTGTCTGActgcagTACAGTAATAAAGAAAC- 3' (SEQ ID NO:12) and NcoI primer), LS3 (5' -AAGCTTGTCTGAGGATTTctgcagAATAAAGAAACGAGGTAG- 3' (SEQ ID NO:13) and NcoI primer), LS4 (5'-AAGCTTGTCTGAGGATTTTACAGTctgcagGAAACGAGGTAGCCCAAA- 3' (SEQ ID NO:14) and NcoI primer), LS5 (5'-AAGCTTGTCTGAGGATTTTACAGTAATAAAActgcagAGGTAGCCCAAACAAG- 3' (SEQ ID NO:15) and NcoI primer).

**Paragraph at Page 21, line 11 to line 21**

[Method 2] The following clones were constructed by inverse PCR using PL1 as a template and LA-Taq polymerase (Takara) along with amplification primers shown in parentheses: PL2 (5' -TCAATGGGACACGCTGCCTGACCACCATGT- 3' (SEQ ID NO:16) and pUC19 primer: 5' -GGCGTAATCATGGTCATAGCTGTTTCCTGTG- 3' (SEQ ID NO:17)),

PL6 (5' -TGTCGGTGCAAAAAATGAAACCCCAAACCTT- 3' (SEQ ID NO:18) and pUC19 primer), PL7 (5' -AATGTTTATCCCTTGCACACATTTTCACATC- 3' (SEQ ID NO:19) and pUC19 primer), PL8 (5'-GCAAAACATCACAACCTCTAGAAAC- 3' (SEQ ID NO:20) and pUC19 primer), PL4C (5' - GTTTGGCTGCAGTCGTTTCTTTATTACTGTAAAATCCTC- 3' (SEQ ID NO:21) and 5' -CAATACTGCAGTATATGTTATGATATAATATGATGCAGC -3' (SEQ ID NO:22)). The amplified fragments were blunt-ended and self-ligated.

**Paragraph at Page 21, line 22 to Page 22, line 10**

[Method 3] To construct the plasmid Pra2-35S90LUC (GF), the upstream region of pra2 was amplified with Pfu DNA polymerase and two primers containing the EcoRV recognition sequence and the PstI recognition sequence, respectively. The amplified DNA fragments were subcloned into the EcoRV site of pZEr0-2.1 (Invitrogen) and digested with EcoRV and PstI. The recovered EcoRV-PstI fragments were subcloned into the EcoR-PstI site of pBI221-LUC+. Five DNA fragments having different lengths were amplified. Namely, the following clones were constructed with amplification primers shown in parentheses: GF1 (GF primer: 5'-TACTGCAGAAAAGTAACACATATTT- 3' (SEQ ID NO:23) and 5' -TGGTGATATTGTTTAGATATCATATTATTGC- 3' (SEQ ID NO:24)), GF2 (GF primer and 5' -ATGATATCCAAGGGATTGGAAT- 3' (SEQ ID NO:25)), GF3 (GF primer and 5' -GTGATATCGGGATAAACATTTTAAGG- 3' (SEQ ID NO:26)), GF4 (GF primer and 5' -TTGATATCCCGACAAAGATCACAC- 3' (SEQ ID NO:27)), GF5 (GF primer and 5' - GGGATATCTCGTTTCTTTATTACT-3' (SEQ ID NO:28)).

**Paragraph at page 25, line 22 to Page 26, line 11**

To determine the core sequence in the cis-element involved in red light-mediated repression of the expression of a reporter gene, said 31-bp region was analyzed by linker scanning. Five DNA fragments having changes in a 6-bp region at different positions were prepared (Fig. 6a). The dark condition and red light treatment condition were the same as the conditions described in Example 6. As a result, LS2 and LS3 did not show red light responsiveness any more (Fig. 6b). Especially, LS3 showed no light responsiveness, indicating the presence of a core sequence in the region where the linker was inserted. All the clones other than LS3 showed light responsiveness. These results show that a 12-bp core sequence (5'-GGATTTTACAGT-3') (SEQ ID NO:1) is present in the phytochrome-responsive cis-element. This 12-bp core sequence is a novel core sequence in phytochrome-responsive cis-elements because it is not present in light- or phytochrome-responsive cis-elements so far reported.

**Paragraph at Page 27, line 20**

WT1 5' -GTCTGAGGATTTTACAGTAATAAAGAAACGA-3' (SEQ ID NO:29)

**Paragraph at Page 27, line 21**

WT2 5' -TCGTTTCTTTATTACTGTAAAATCCTCAGAC-3' (SEQ ID NO:30)

**Paragraph at Page 28, line 7**

MT1 5' -GTCTGAGGCTTTTCCCGTAATAAAGAAACGA-3' (SEQ ID NO:31)

**Paragraph at Page 28, line 8**

MT2 5' -TCGTTTCTTTATTACGGGAAAAGCCTCAGAC-3' (SEQ ID NO:32)

**Paragraph at Page 29, line 6 to line 24**

At first, CaMV 35S46 was amplified by PCR under the following conditions. PCR reaction was performed using the pBI221-LUC+ vector as a template along with primer 35S46UP (5'-AAGCTTGGATCCCTCGAGCTGCAGGATATCGCAAGACCCTTCCTCTATATAAGGA- 3') (SEQ ID NO:33) and primer KZ35SDW (5' -TTCCATGGAAAGCTGCCTAGGAGATCCTCT- 3') (SEQ ID NO:34) and the PCR product was subcloned into the pZER0-2 vector. A plasmid was purified from the resulting clone and then treated with the restriction endonucleases HindIII and NcoI to recover the fragment of interest CaMV 35S46. CaMV 35S46 was inserted into the pBI221-LUC vector digested with HindIII and NcoI to give a vector 35S46-LUC. However, this vector contained a single nucleotide change as compared with the 35S promoter of the initial pBI221 vector because the HindIII site near the translation initiation point of the luciferase gene in the pBI221-LUC+ plasmid was removed by using the KZ35SDW primer. The nucleotide sequence of the promoter region amplified by PCR was confirmed by sequencing.

**Paragraph at Page 29, line 25 to Page 30, line 8**

An oligonucleotide WT3 (5'-TGAGGATTTTACAGTAATTGAGGATTTTACAGTAATTGAGGATTTTACAGTAA T-3') (SEQ ID NO:35) having three 18-bp sequences including 3 base pairs added at each end of the 12-bp cis-element was synthesized and phosphorylated at the 5' end and then ligated as a single strand. Then, WT4 (5'-ATTACTGTAAAATCCTCAATTACTGTAAAATCCTCAATTACTGTAAAATCTCA-3') (SEQ ID NO:36) complementary to WT3 was phosphorylated at the 5'-end and then annealed to said WT3 which had been ligated as a single strand, and the annealed product was inserted into the EcoRV site of pZEr0-2 (Invitrogen) to give a plasmid containing 9 copies of the 18-bp sequence.

**Paragraph at Page 29, line 9 to line 24**

To remove the sequence derived from the pZEr0-2 vector, PCR was performed using said plasmid containing 9 copies of the 18-bp sequence as a template along with primer 18X9RMDW (5' - GCGATATCCTGGATCCTGAGGATTTT- 3') (SEQ ID NO:37) and primer 18X9RMUP (5' -AGCGGCCGCCAGTGTGGATATCATTACTGT- 3') (SEQ ID NO:38) having a BamHI site and an EcoRV site, respectively. The amplified fragment was digested with BamHI and EcoRV and inserted into the BamHI-EcoRV site of the 35S46-LUC vector to give pGF9 shown in Fig. 9a. The sequence of the region amplified by PCR was determined by sequencing. Then, a plasmid pGF9M in which three adenines in the 12-bp cis- element of pGF9 are replaced by cytosines was constructed in the same manner as described above by using primer MT3 (5' - TGAGGCTTTTCCCGTAATTGAGGCTTTTCCCGTAATTGAGGCTTTTCCCGTA

AT-3') (SEQ ID NO:39) and primer MT4 (5'-

ATTACGGGAAAAGCCTCAATTACGGGAAAAGCCTCAATTACGGGAAAAGCC

TCA- 3') (SEQ ID NO:40).